8.—Some Preliminary Experiments on Adrenal Function during Seasonal Stresses in a Wild Marsupial (Setonix brachyurus)

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Introduction

An effort is being made by a group of workers in Western Australia to assess the clinical conditions of an island population of the herbivorous ruminant wallaby *Setonix brachyurus* during various seasons of the year (Main, Shield and Waring 1959; Hodgkin and Sheard 1959). On Rottnest Island the animal is protected by fauna laws and has no predators, so an excellent opportunity is presented for analysing causes of death and hence population control. Much work on the individual physiology of the animal has been carried out. It has been established, from skull and dentition data, that death occurs almost entirely in late summer. Analysis of the protein content of the diet gives a strong indication that the seasonal fall in plant protein in late summer is a major agent in precipitating death, but other aspects such as availability of free water, trace elements, etc. are under consideration. Whatever the primary cause of the general seasonal debility (and death of some individuals) there is little doubt that in late summer animals are exhibiting stress as envisaged in the Selye syndrome. Under these circumstances it is of interest to enquire how adrenal function varies at different seasons and this paper reports some preliminary experiments along these lines.

Analysis of adrenal steroids in the adrenal vein would clearly be the method of choice, but at present there is no way of doing this under field conditions. Roberts (1957) has shown that there is usually correspondence between ability of an extract to stain steroid secretions and to deplete ascorbic acid, but the correspondence is not precise. Nevertheless, a valuable indication of steroid secretion, in the absence of its direct measurement, can be obtained from measuring ascorbic acid content, which is practicable in the field. Therefore, ascorbic acids were measured. The fat content, histologically determined, was estimated to see if it correlated with the ascorbic acid content.

Materials and Procedure

Only females were used and they were selected for similarity of history—area where collected, length of time in captivity, care and feeding. They were weighed at the beginning of the experiment. Some animals were killed for use as normal controls. Others were kept with feed (sheep nuts, 11% protein) but no water and then periodically animals were killed in varying stages of dehydration.

All animals were killed by cervical dislocation after which the adrenal glands were promptly removed. The glands were weighed individually to the nearest milligram with a torsion balance. The right one was used immediately for ascorbic acid determination and the left one was cut in two with one half being placed in Bouin's fixitive for histological study and the other in 10% formalin for fat determination. The weights of liver, spleen, and in some cases of the empty stomach were recorded. The right adrenal gland was assayed for ascorbic acid content using the procedure described by Hawk, Oser and Summerson (1947, p. 1137). Readings were made with a Bausch and Lomb Spectronic 20 colorimeter. In retrospect it is realised that these should have been matched against weighed quantities of pure ascorbic acid to achieve a reference standard. This was not done but the figures afford a reliable comparative measure of the samples. The tissues placed in Bouin's fixitive were sectioned and stained with Ehrlich's haematoxylin. Those fixed in formalin were sectioned on a freezing microtome and sections were stained with Sudan III and IV for fat determination.

Another phase of the study involved observations on animals from Rottnest Island which had not been in captivity previously. Groups of animals were collected from the "North Road" area where there was no known source of drinking water and are referred to here as the "Rottnest dry" animals. Others were collected from the "Serpentine" area where there were sources of drinking water and are known as "Rottnest wet" animals (map in Hodgkin and Sheard, 1959). All Rottnest animals were collected at night using hand nets and were held in bags until the following day. The processing of the animals was similar to that of the yard animals except that the right adrenal glands were placed in plastic vials and frozen in a thermos jar of dry ice (solid CO_2). Ascorbic acid determinations were made the following day.

In determining adrenal fat, sections of the cortex were, by observation, divided into four equal regions which were numbered from the outer to the inner zones (Fig. 1). Scores were assigned to each area from one to four upon the estimated density of the Sudan stain. The sum of these gave the "score" for that slide. Two separate slides, stained at different times, were evaluated and scores were compared. There was close agreement between the two slides.

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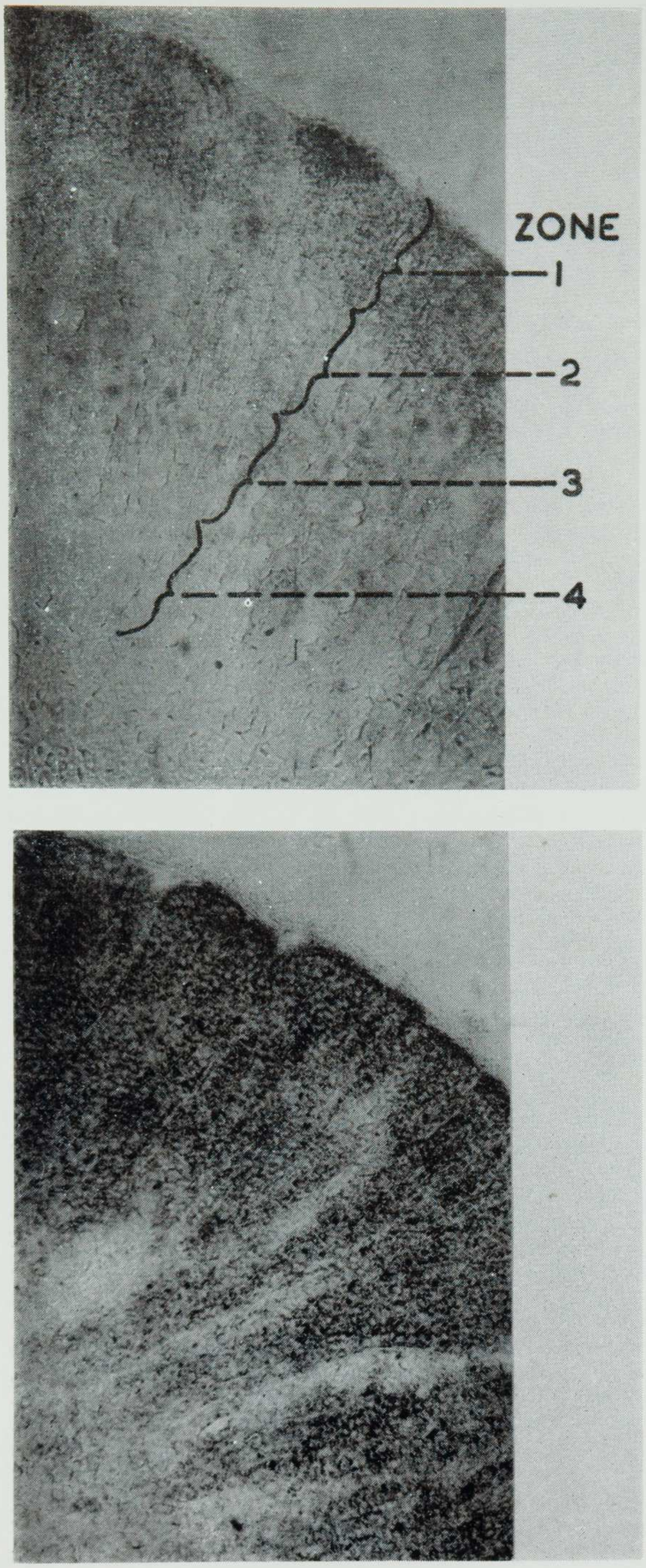

Fig. 1.—Photomicrographs of sections of adrenal glands with Sudan staining to show fat. Upper section with little fat giving a score of 2-1-1-0. Lower section with high fat with a score of 4-3-2-1.

Results

One group of nine quokkas was placed in a large yard without water but with limited green vegetation growing and with dry feed (sheep nuts) available. These animals had previously been confined to pens for several weeks with unlimited feed and water. Animals were killed at intervals. During the three to four weeks that some green vegetation was available, there was little loss of body weight. Results from adrenal ascorbic acid determinations indicated a rise in the earlier stages of dehydration when compared with animals receiving water. Following this initial rise the ascorbic acid level dropped and continued to do so as long as the animals survived without water. Three animals in the group were returned to feed and water after dehydration and within two weeks on full feed and water the ascorbic acid was determined to be at normal levels (Fig. 2).

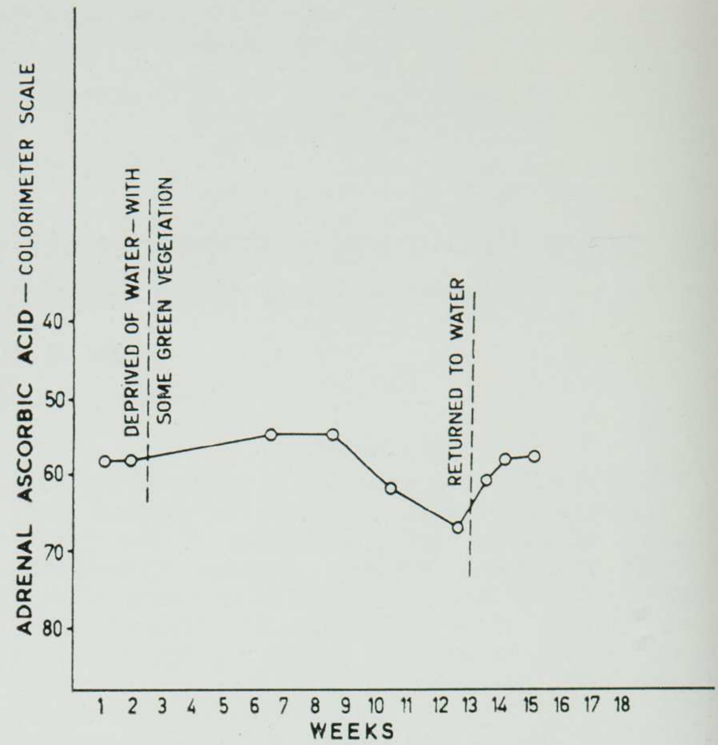

Fig. 2.—Levels of adrenal ascorbic acid in animals deprived of water. Some green vegetation was growing in pen for the first five to six week. After the 13th week, three animals were returned to water.

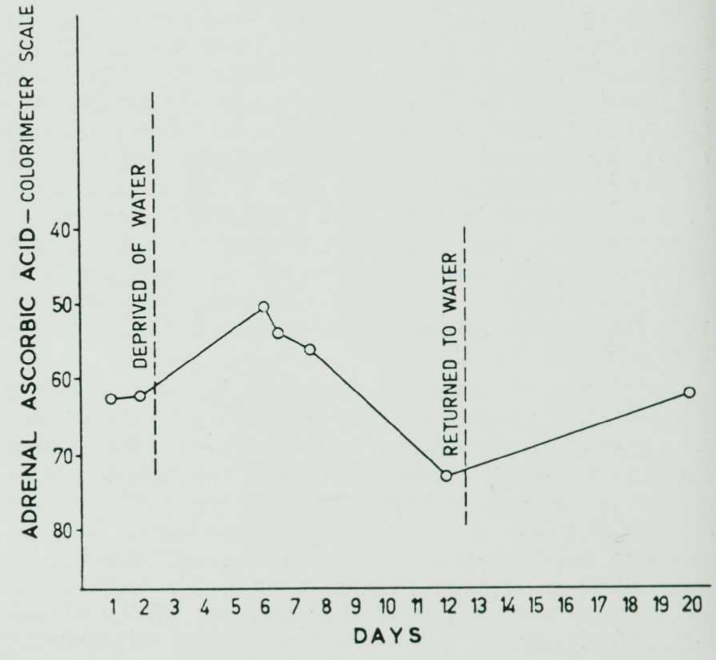

Fig. 3.—Levels of adrenal ascorbic acid in animals with conditions similar to those in Fig. 2 except no green feed was available from the start.

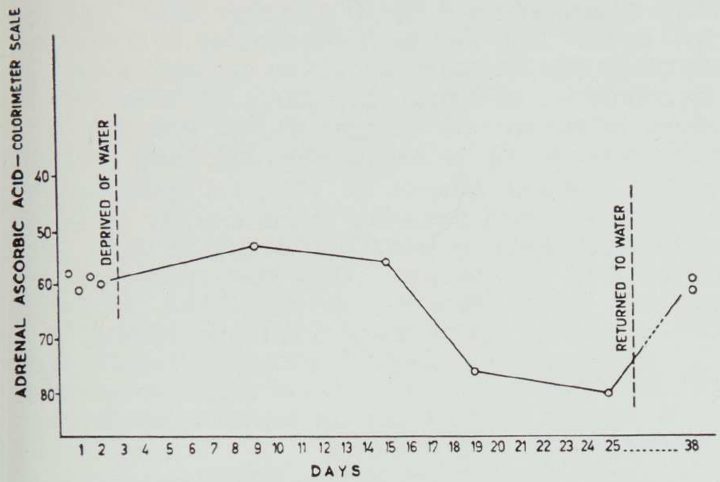

Fig. 4.—Levels of adrenal ascorbic acid in animals with conditions as in Fig. 3. Determinations were made on four animals before dehydration, four during dehydration and on two after being returned to water.

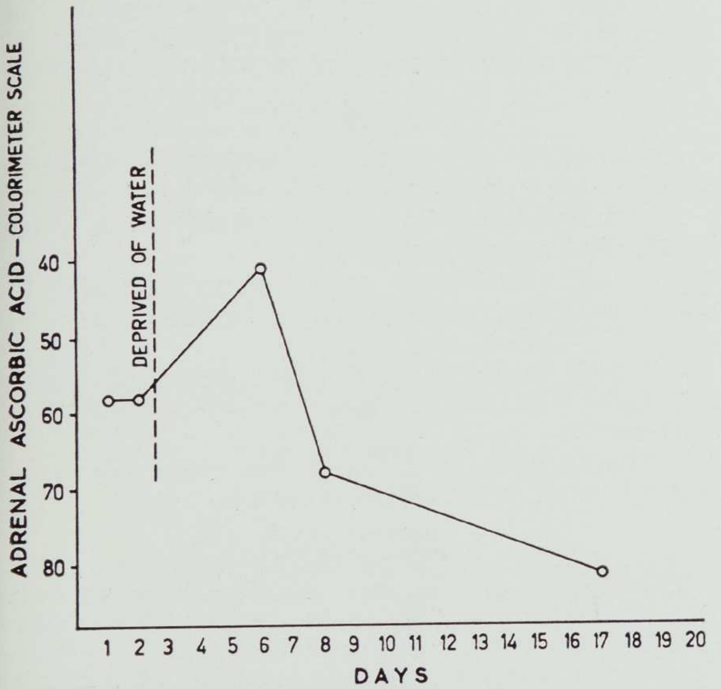

Fig. 5.—Levels of adrenal ascorbic acid in animals brought from a dry area of Rottnest Island and held without water.

A second group of seven animals was deprived of water and there was no green vegetation in the yard. These animals as in the first group had previously been held in pens with water and feed. Dry feed (sheep nuts) was available. As in the first group, there was a rise in adrenal ascorbic acid followed by a drop to low levels and finally a rise to normal after a return to water of approximately one week (Fig. 3).

The third group of ten animals subjected to experimental conditions as in group two, gave essentially the same pattern of adrenal ascorbic acid as in previous trials (Fig. 4).

Group number four consisted of animals brought from the "dry" area of Rottnest Island which were placed in a pen without water. These animals showed prompt changes in adrenal ascorbic acid levels and in a pattern similar to that of other groups but with the changes coming more quickly (Fig. 5). One animal died before examination.

It being established that (a) severe dehydration causes elevation, and then depletion, of adrenal ascorbic acid content (Figs. 2-4) and by (b) animals from "dry" areas on the island, although having approximately the same level

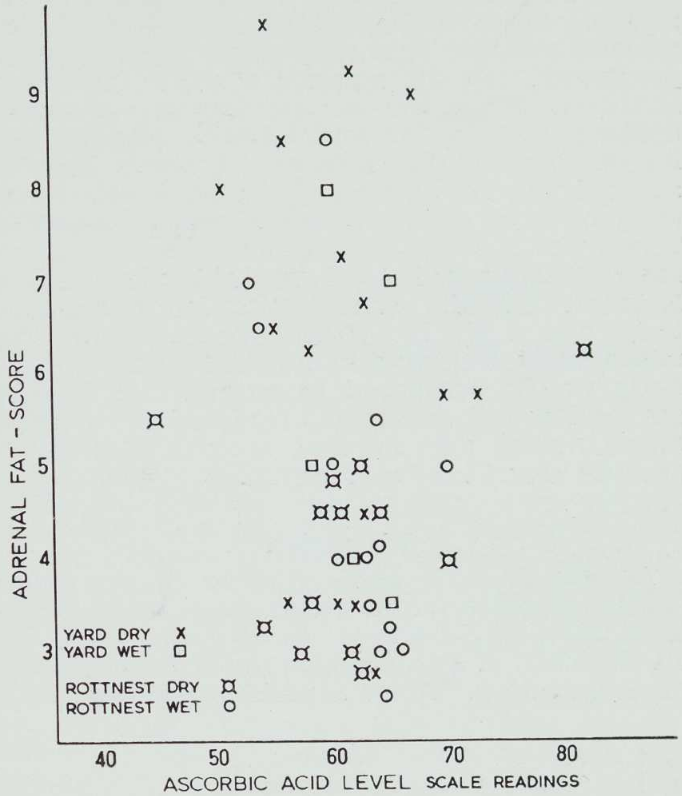

Fig. 6.—Adrenal ascorbic acid levels and adrenal fat scores showing no significant correlation between these factors. There was evidence, however, that Rottnest animals had less adrenal fat than animals kept in captivity.

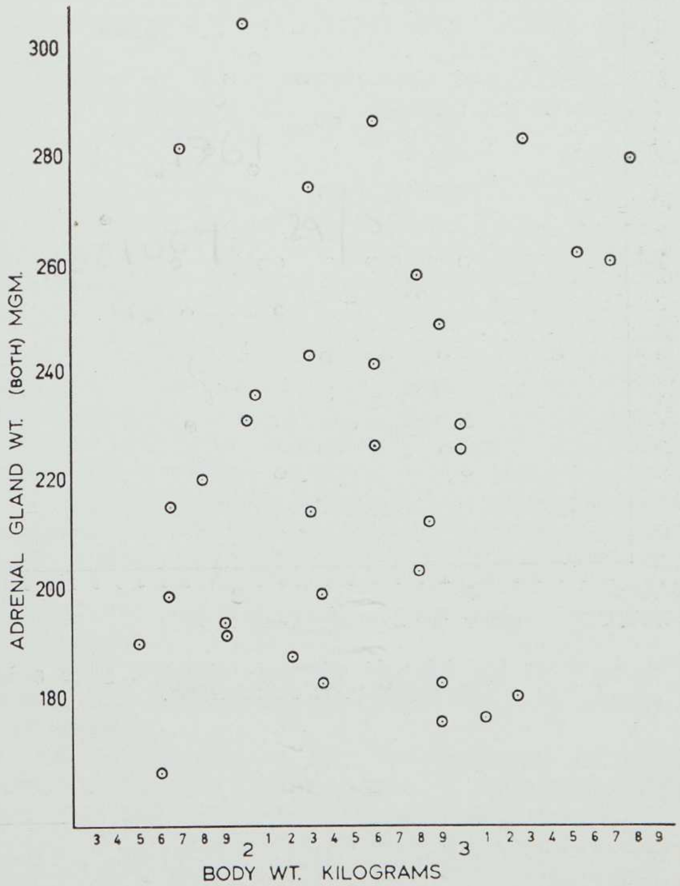

Fig. 7.—Body weight and adrenal gland weight gave only a low degree of correlation.

of ascorbic acid at the beginning of the experiment, showed a more dramatic ultimate depletion on complete dehydration (Fig. 5), an effort was made to get more animals from a "dry" area to see whether ascorbic depletion occurred in the wild during the low rainfall season. The animals captured had approximately the same adrenal ascorbic acid content as those captured previously. In all animals studied (yard and Rottnest) there was no significant correlation between adrenal fat and adrenal ascorbic acid levels. There was, however, a lower level of adrenal fat in animals from Rottnest Island than in most of the animals kept in captivity. There was no recognizable difference in adrenal fat in animals from the wet and dry areas of Rottnest Island. With all animals included, there was a low but significant correlation between body weight and adrenal gland weight. Body weight compared to adrenal fat likewise gave a low but significant correlation. Adrenal gland weight and adrenal ascorbic acid levels gave no significant correlation.

Discussion

Among animals kept in pens there was a consistent pattern of adrenal gland ascorbic acid level upon dehydration. In all cases there was an elevation of ascorbic acid in the earlier stages of dehydration. After a peak production, however, there was a drop in ascorbic acid that did not again rise during dehydration. Upon returning the animals to water in any stage of dehydration, adrenal ascorbic acid returned to usual levels in one to two weeks and this was accompanied by a rapid gain in body weight.

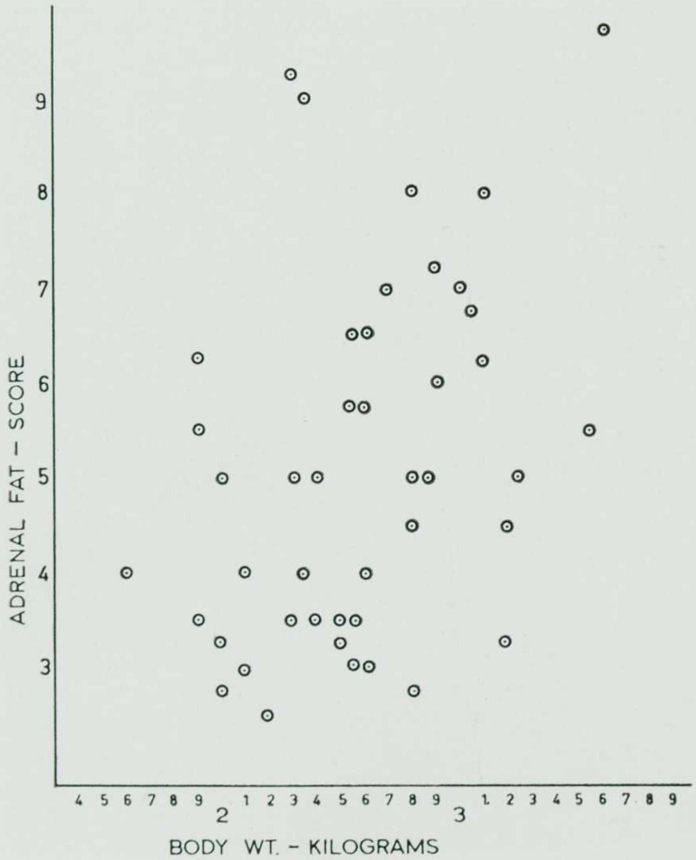

Fig. 8.—Body weight and adrenal fat score gave a low but significant correlation.

The adrenal glands of wild animals caught on Rottnest had ascorbic levels similar to those of animals held in pens and supplied with adequate feed and water. This was true even for animals taken from dry areas at the height of the summer. This may seem surprising but: (a) the summer of 1958 was not harsh and, (b) it is likely that the more vigorous animals were caught because on initial disturbance the weaker animals do not come out. Whatever the interpretation may be, it was demonstrated that severely dehydrated captive animals have a lowered adrenal ascorbic acid titre and that none of the wild ones taken had reached this level.

In view of the above it would plainly be valuable to test a larger number of animals, and particularly at the end of a harsh summer.

Summary

1. In the early stages of dehydration quokkas show a rise in adrenal ascorbic acid.

2. Continued dehydration results in a drop in adrenal ascorbic acid.

3. Upon returning dehydrating animals to water, adrenal ascorbic acid levels return to normal.

4. Wild animals from "dry" areas of Rottnest Island in 1958 did not show altered ascorbic acid levels.

5. Quantities of adrenal fat were not cerrelated with ascorbic acid levels.

Acknowledgments

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